

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Kim

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Group Art Unit: 1639

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Examiner: STEELE, Amber D.

For: CHIP PRODUCTION, HYBRIDIZATION AND DATA INTERPRETATION
FOR ANTIBODY AND PROTEIN MICROARRAYS

Attorney Docket No: 3087.00024

PRELIMINARY AMENDMENT

Dear Sir:

This is in response to the Office Action dated July 19, 2010, part of Paper No./Mail Date 20100629. Applicant hereby petitions for a two-month extension of time within which to respond to the outstanding Office Action. Granting the petition would extend the time for filing the Response to December 19, 2010. The appropriate petition fee of \$245.00 is hereby authorized to be charged to our Deposit Account No. 11-1449.

Please enter the following amendments to the application in order to place the application in condition for allowance. Please amend the above-captioned patent application consistent with the instructions found attached hereto:

IN THE CLAIMS:

1. (Currently Amended) An antibody microarray screening method including the steps of depositing a plurality of spots comprising ~~[[of]]~~ antibodies selected from the group consisting of purified immunoglobulins, ~~[[and]]~~ fluids that are unprocessed for immunoglobulin isolation, and combinations thereof on predetermined positions on a substrate; hybridizing the plurality of spots of antibodies with a plurality of labeled target proteins; quantifying the label signal strength produced at each spot; quantifying levels of target protein captured at each spot on the basis of label signal strength; and comparing levels of target protein among the plurality of samples of labeled target proteins.

2. (Currently Amended) The antibody microarray screening method according to claim 1, wherein the ~~step of depositing a plurality of spots of antibodies is further defined as depositing a plurality of spots of antibodies~~ are specific for proteins selected from the group consisting of drug-metabolizing enzymes and proteins functionally related with said drug-metabolizing enzymes.

3. (Currently Amended) The antibody microarray screening method according to claim 2, wherein the ~~step of depositing a plurality of spots of antibodies specific for proteins selected from the group consisting of drug-metabolizing enzymes~~ are ~~and proteins functionally related with said drug-metabolizing enzymes is further defined as depositing a plurality of spots of antibodies specific for cytochromes P450.~~

4. (Currently Amended) The antibody microarray screening method according to claim 2, wherein ~~said the step of depositing a plurality of spots of antibodies specific for proteins functionally related with said drug-metabolizing enzymes~~ are ~~is further defined as depositing a plurality of spots of antibodies specific for at least one~~ protein enzyme selected from the group consisting of mitochondrial proteins, apoptosis-related proteins, anti-oxidant proteins,

oxidative stress proteins, and intracellular protein degradation proteins.

5. (Currently Amended) The antibody microarray screening method according to claim 1, wherein the ~~step of depositing a plurality of spots of antibodies on predetermined positions on a~~ substrate is further defined as ~~depositing a plurality of spots of antibodies on predetermined positions on a~~ polyacrylamide-based hydrogel (~~polyarylamide-based~~) coating.

6. (Previously Presented) The antibody microarray screening method according to claim 1, further including the step of determining optimal spotting concentrations of said purified immunoglobulins and the optimal dilution factors of said fluids that are unprocessed for immunoglobulin isolation.

7. (Currently Amended) The antibody microarray screening method according to claim 1, wherein the ~~step of depositing a plurality of spots of antibodies selected from the group consisting of purified immunoglobulins and fluids that are unprocessed for immunoglobulin isolation is further defined as~~ depositing a plurality of spots of antibodies selected from the group consisting of ~~purified immunoglobulins and fluids that are selected from the group consisting of~~ ascites fluids, hybridoma culture medium, and anti-sera.

8. – 11. (Canceled)

12. (Withdrawn) An antibody microarray screen comprising: a substrate; monoclonal antibodies as purified immunoglobulins, wherein said antibodies are spotted on predetermined positions on said substrate; ascites fluid spotted on said substrate; and hybridoma culture media spotted on said substrate, wherein said ascites fluid and hybridoma culture media with higher amounts of proteins than said purified immunoglobulin proteins to compensate non-immunoglobulin proteins in said unprocessed fluids are spotted on predetermined positions on said substrate.

13. (Withdrawn) The antibody microarray screen according to claim 12, wherein said monoclonal antibodies detect proteins selected from the group consisting of drug-metabolizing enzymes, cytochromes P450, and oxidative stress proteins.

14. (Withdrawn) The antibody microarray screen according to claim 12, wherein said substrate includes a hydrogel (polyarylamide) coating.

15. (Withdrawn) The antibody microarray screen according to claim 12 and determining optimal spotting concentrations of IgG by further comprising steps of (a) hybridizing the slides with labeled secondary immunoglobulins, (b) scanning and quantitating signal strength of each spot, and (c) selecting optimal concentrations of IgG using %visible IgG spots.

16. (Withdrawn) A method of manufacturing an antibody microarray comprising the step of spotting more than a single concentration of antibodies on a microarray substrate to increase the number of up-regulated protein detection.

17. (Withdrawn) The method according to claim 16, wherein the antibody concentration is more than 5 µg/ml IgG.

18. (Withdrawn) An internal control molecule for use in an antibody microarray comprising a protein, wherein said protein is unexpressed in the array sample for normalization of focused (non-global) array data.

19. (Withdrawn) The internal control molecule according to claim 18, wherein said protein is selected from the group consisting of a Flag protein and a non-mammalian protein.

20. (Withdrawn) The internal control molecule according to claim 18, wherein the internal control molecule is used to compare the expression ratio of

house-keeping proteins to select housekeeping genes by determining any difference between the control and experimental samples.

21. (Withdrawn) A method of determining optimal spotting concentrations of IgG comprising the steps of: (a) spotting increasing concentrations of IgG on microarray slides; (b) hybridizing the slides with secondary IgG with a detectable signal; and (c) scanning and quantitating signal strength of each spot and selecting optimal concentrations of IgG.

22. (Withdrawn) A method to increase a detectable signal with microarray analysis comprising the steps of using an intensive molecular signal, wherein the intensive molecular signal is produced by conjugation of a dye and a reporter molecule to a protein whereby interference of IgG binding to a protein is created.

23. (Withdrawn) The method according to claim 22, wherein the intensive molecular signal is produced by conjugation of a dye and a reporter molecule to a protein to the extent that interference of Coomassie blue stain binding to the protein is created.

24. (Withdrawn) The method according to claim 22, wherein the intensive molecular signal is used for antibody microarrays.

25. (Withdrawn) The method according to claim 22, wherein the intensive molecular signal is used for protein microarrays.

26. (Withdrawn) A method to increase a detectable signal with microarray analysis comprising the steps of: conjugating of a dye and a reporter molecule to a protein; and creating interference of an IgG molecule binding to the protein.

27. (Withdrawn) A method of producing antibody microarrays comprising the steps of spotting antibodies for Phase I and II drug metabolizing enzymes and proteins functionally related with the drug-metabolizing enzymes on a microarray substrate.

28. (Withdrawn) The method according to claim 27, wherein the proteins functionally related with drug-metabolizing enzyme are selected from the group consisting of mitochondrial proteins, apoptosis-related proteins, anti-oxidant proteins, oxidative stress proteins, and intracellular protein degradation proteins.

29. (Withdrawn) The method according to claim 27, wherein the targeted drug-metabolizing enzyme antibody microarray includes an internal control to be used for data normalization.

30. (Withdrawn) The method according to claim 29, wherein the internal control is a Flag protein.

31. (Previously Presented) The antibody microarray screening method according to claim 6, wherein the step of determining optimal spotting concentrations of said purified immunoglobulins and said fluids that are unprocessed for immunoglobulin isolation further includes the steps of depositing, at a first set of locations, a plurality of spots of at least one purified immunoglobulin at a plurality of concentrations; depositing, at a second set of locations, a plurality of spots of at least one fluid that is unprocessed for immunoglobulin isolation at a plurality of dilution factors; hybridizing the spots with labeled secondary immunoglobulins; scanning and quantifying the signal strength of label at each spot; selecting an optimal concentration of the at least one purified immunoglobulin on the basis of the production of an optimal label signal strength; and selecting an optimal dilution factor of the at least one fluid that is unprocessed for immunoglobulin isolation by selecting a dilution factor that yields a label signal strength similar to that produced by the optimal

concentration of the at least one purified immunoglobulin.

32. (Previously Presented) The antibody microarray screening method according to claim 31 wherein the step of scanning and quantifying the signal strength of label at each spot is further defined as scanning and determining the percentage of visible spots in the scanned image.

33. (Previously Presented) The antibody microarray screening method according to claim 1 wherein the step of depositing a plurality of antibody spots on predetermined positions on a substrate is further defined as spotting fluids that are unprocessed for immunoglobulin isolation at higher amounts of protein than purified immunoglobulins, thereby compensating for the lower concentrations of antigen specific immunoglobulins in said fluids that are unprocessed for immunoglobulin isolation.

34. (Previously Presented) The antibody microarray screening method according to claim 32 wherein the step of depositing a plurality of antibody spots on predetermined positions on a substrate is further defined as spotting fluids that are unprocessed for immunoglobulin isolation at a 10 – 1000 fold dilution and spotting purified immunoglobulins at a protein concentration of 5-500 µg/ml.

REMARKS

Claims 1-7 and 12-34 are currently pending in the application. Claims 12-30 are withdrawn. Claim 1 is the only claim in independent form. Claims 8-11 have been canceled.

Claim 5 is objected to for informalities, and Applicant has amended the claim as suggested by the Office Action to read “polyacrylamide-based hydrogel coating”. Reconsideration of the objection is respectfully requested.

Claims 2-5 and 7 are objected to for informalities in that the claim language is excessive and redundant for reiterating the method step of deposition. In response thereto, claims 2-5 and 7 have been amended to remove excess language and to clarify the subject matter, based on the example given for claim 2 by the Office Action. Reconsideration of the objection is respectfully requested.

Claims 1-7 and 31-34 stand rejected under 35 U.S.C. §112, first paragraph, as failing to comply with the written description requirement. Specifically, the Office Action holds that support for the claim amendments received on December 22, 2009, was not found in the originally filed disclosure. In response thereto, the following is support for each of the claim amendments in the following paragraphs and figures:

“hybridizing the plurality of spots of antibodies with a plurality of labeled target proteins”

[0024] Generally, the present invention relates to a microarray including a substrate on which a ***plurality of sample spots*** is arranged in a two-dimensional array. The present invention provides a method of producing antibody drug-

metabolizing enzyme antibody microarrays. Specifically, the present invention relates to microarrays including closely related cytochromes P450. The present invention also relates producing antibody chips with fluids that are not processed for immunoglobulin isolation including anti-sera, ascites fluids, and hybridoma culture media. The present invention relates to analysis of levels of spotted IgG by hybridization of labeled secondary IgG to decide optimal antibody levels to spot on the slide. Use of a spiked internal control to normalize microarray data obtained from focused (non-global) array analysis is also disclosed. Further, the present invention provides a method to increase signal with array analysis using an intensive molecular signal.

[0026] The present invention is useful in toxicoproteomics. Specifically, the present invention can be used in the identification, quantification, and qualification of **protein products**. Further the present invention is useful in analyzing numerous samples simultaneously. Automated chip construction, use of fluorescent signals, and custom digital image analysis make it possible to monitor expression of hundreds of proteins and to obtain expression profiles of environmental toxins. Additionally, the present invention can predict optimal ranges of antibody spotting levels by hybridization of the antibodies on the slide with secondary antibodies with known signal strength. The present invention can be used to analyze levels of spotted IgG by hybridization of labeled secondary IgG to determine optimal antibody levels to spot on the slide.

[0030] The present invention has numerous embodiments. In one embodiment, the present invention provides an antibody microarray screen including a substrate, monoclonal and polyclonal antibodies that are purified immunoglobins, and fluids unprocessed for immunoglobulin isolation. The antibodies and fluids are spotted on predetermined positions on the substrate. ***The antibodies detect numerous proteins. For example, drug-metabolizing enzymes such as cytochromes P450, proteins functionally related with the drug-metabolizing enzymes such as mitochondrial proteins, apoptosis-related proteins, anti-***

oxidant proteins, oxidative stress proteins, and intracellular protein degradation proteins, and other similar proteins known to those of skill in the art can all be used with the microarray of the present invention. Further, the substrate can include a hydrogel (polyarylamide-based) coating. The screen can optionally include labeled secondary immunoglobulins, wherein the secondary immunoglobulin levels are analyzed. Finally, the screen includes fluids such as, but not limited to, ascites fluids, hybridoma culture medium, anti-sera, and the like.

[0090] The hydrogel chips were blocked overnight at 4.degree. C. with 1% BSA/PBS blocking solution. The rat hepatic proteins were dialyzed with 10 kDa molecular cut-off membrane: An internal control protein, Met-Flag fusion proteins of E. coli bacterial alkaline phosphatase recognized by anti-Flag (Sigma Co.) was added to both untreated and PB-treated samples prior to Cy3 or Cy5 conjugation. The rat proteins (1 mg) with Flag protein (2 .mu.g) were mixed and cross-linked with NHS-Cy3 or NHS-Cy5 (Amersham) (0.2 mg) according to the manufacturer's instructions. **The probes [referring to the antibodies]** were washed three times with 500 .mu.l PBS and concentrated to 40 .mu.l using microconcentrator spin columns (Amicon, 10 kDa cut-off). As described above for Control 1/PB 1 set (1st set) and Control 2/PB 2 set (2nd set), **hybridization of the probes** with dye swaps (in total, 4 hybridizations, 16 data points for each IgG concentration) was carried out for four hours at room temperature in Detroit R&D hybridization buffer (total volume, 750 .mu.l). **The hybridization solution contained 2 mg of rat liver protein [i.e. plurality of target labeled protein].** A representative image is shown in **FIG. 1**, "Experiment."

"quantifying the label signal strength produced at each spot"

"quantifying the levels of target protein captured at each spot on the basis of label signal strength"

[0075] After hybridization, the chips were dried and images were scanned at 532 nm (green) and 635 nm (red) and pseudo-colored images were obtained using a GenePix 4000A (Axon Instruments). ***Signal intensity of each target protein was quantified*** with GenePix software using a gal (spot design) file, which contained the identity of each protein and its location in the 384-well plate.

“comparing levels of target protein among the plurality of samples labeled target proteins”

[0076] Consolidated data from four microarray analyses were obtained with 0.5, 5, 50 and 500 .mu.g/ml antibody spots (16 data points per spot). ***Fold-increase or decrease in protein expression levels after phenobarbital treatment of rats was calculated*** by combining data obtained by dye swaps using GeneSpring analysis. Signal levels under 10 (an arbitrary unit by GenePix quantitation) in both control and phenobarbital treated samples were deleted.

[0077] The mean (normalization factor) of 3 Flag protein internal control data points for each IgG concentration was obtained. Data points were normalized by dividing fold-increase or decrease in protein expression levels with the normalization factor pertinent to IgG concentration.

With respect to the method steps of claims 6, 31, 32, 33, and 34, Applicant notes that these claims are identical to withdrawn claim 21 except for the selection of optimal concentration of IgG by % visible IgG spots. New claims 31-34 are supported by the specification in the following paragraphs as well as Table 2:

[0039] In a further embodiment of the present invention, polyclonal IgG, anti-sera and monoclonal antibodies purified or as ascites fluids were used to produce a drug metabolizing enzyme antibody microarray (Table 1). The fluids used for slide spotting without immunoglobulin fraction purification were 10-fold diluted to

achieve array results similar to 0.5 mg/ml IgG. Protein levels of four polyclonal anti-sera ranged from 38 to 75 mg/ml. Thus, when anti-sera or ascites fluids were spotted, higher amount of protein compared with purified IgG protein has to be spotted to compensate non-IgG proteins in the fluids.

[0040] A method to analyze quality of the array spots has been developed with the present invention. The antibody slide was hybridized with labeled secondary IgG, e.g. 25 .mu.g Cy3-conjugated anti-rabbit IgG mixed with 50 .mu.g Cy5-conjugated anti-mouse IgG, followed by scanning of the chip to compare signal strength of each spot and to assess evenness of each spot (Table 2).

[0041] Signal levels obtained from spotting 100-fold and 10-fold diluted anti-sera or ascites fluids were similar to the spots obtained with 50 and 500 .mu.g/ml IgG spotting, respectively. This result demonstrated that estimation of IgG levels of the anti-sera and ascites fluids was correct. Polyclonal antibodies for CYP3A were spotted for array analysis as anti-serum without isolation of IgG. CYP3A was previously reported to increase after phenobarbital treatment. Array analysis revealed that CYP3A expression increased higher than two-fold after phenobarbital treatment (Table 3). Six mouse ascites fluids including antibodies for tubulin-alpha demonstrated a 1.7-fold increase after phenobarbital treatment, while alpha-actin (0.8-fold of control, 20% decrease) and beta-actin (1.6-fold increase) were also successfully used for antibody array production (Tables 1 and 4). The results demonstrated that anti-sera or ascites fluids (without IgG purification, thus containing high levels of proteins) could be spotted on the chip to achieve a successful antibody microarray analysis.

“At least one enzyme”

See Table 1, which showed 4 mitochondrial proteins, 13 apoptosis-related proteins, 3 anti-oxidant proteins, 21 oxidative stress proteins, and 2 intracellular protein degradation proteins. At least one enzyme was included to have 5

different groups of proteins in Table 1 functionally related with the drug-metabolizing enzymes.

“10-1000 fold dilution” and “5-500 $\mu\text{g/ml}$ ”

[0040] A method to analyze quality of the array spots has been developed with the present invention. The antibody slide was hybridized with labeled secondary IgG, e.g. 25 μg Cy3-conjugated anti-rabbit IgG mixed with 50 μg Cy5-conjugated anti-mouse IgG, followed by scanning of the chip to compare signal strength of each spot and to assess evenness of each spot (Table 2).

[0041] Signal levels obtained from spotting 100-fold and 10-fold diluted anti-sera or ascites fluids were similar to the spots obtained with 50 and 500 $\mu\text{g/ml}$ IgG spotting, respectively. This result demonstrated that estimation of IgG levels of the anti-sera and ascites fluids was correct. Polyclonal antibodies for CYP3A were spotted for array analysis as anti-serum without isolation of IgG. CYP3A was previously reported to increase after phenobarbital treatment. Array analysis revealed that CYP3A expression increased higher than two-fold after phenobarbital treatment (Table 3). Six mouse ascites fluids including antibodies for tubulin- α demonstrated a 1.7-fold increase after phenobarbital treatment, while α -actin (0.8-fold of control, 20% decrease) and β -actin (1.6-fold increase) were also successfully used for antibody array production (Tables 1 and 4). The results demonstrated that anti-sera or ascites fluids (without IgG purification, thus containing high levels of proteins) could be spotted on the chip to achieve a successful antibody microarray analysis.

[0042] Antibody concentration-dependent signal strength can be obtained by hybridization of secondary IgG utilizing the present invention. Antibodies for the microarray were spotted in four blocks (two spots in each concentration) on top of the slide and the four blocks were repeated on the bottom of the slide. Quality control analysis was carried out using labeled secondary antibodies hybridized

with the antibody chips (FIG. 1, "Quality Control"). Majority of the spots visible in the quality control analysis were round and bright red (monoclonal IgG) or green (polyclonal IgG) with low background signal. Spots in block one (144 spots) were used to count % visible spot counting in each concentration of IgG and shown in Table 2. All spots of 500 $\mu\text{g/ml}$ IgG showed strong signal, 11% and 72% of spots from 50 $\mu\text{g/ml}$ and 5 mg/ml IgG, respectively, failed to show. None of the 0.5 mg/ml IgG spot showed any signal. The result demonstrated that the quality control spot signal strength of each spot was IgG concentration-dependent. Thus, the quality control analysis can be used for selection of optimal IgG concentration for array production.

After proving that 5-500 $\mu\text{g/ml}$ of IgG concentrations had to be translated to 10-1000 fold diluted unprocessed antiserum in paragraphs [0040]-[0041] and Table 2, visible concentrations of IgG or anti-sera were discussed in paragraph [0042]. None of the 0.5 $\mu\text{g/ml}$ IgG spot showed any signal. Thus, 5-500 $\mu\text{g/ml}$ is the range of antibody concentration for spotting. It should be noted that based on paragraph [0041] and Table 2, if 50 $\mu\text{g/ml}$ is 100 fold dilution, the bottom range of 5 $\mu\text{g/ml}$ is 1000 fold dilution. Thus, there is support for 10-1000 fold dilution.

Reconsideration of the rejection under 35 U.S.C. §112, first paragraph, is respectfully requested.

Claims 1-7 and 31-34 stand rejected under 35 U.S.C. §112, second paragraph, as being indefinite. The Office Action holds that it is unclear how spots of antibodies can be purified immunoglobulins or fluids. In response thereto, claim 1, upon which all other claims depend, has been amended to specify that the plurality of spots comprises antibodies that are purified immunoglobulins or fluids. In other words, the antibodies are of the type of purified immunoglobulins or fluids, and purified immunoglobulins and fluids cannot be used if they are not antibodies. Reconsideration of the rejection under

35 U.S.C. §112, second paragraph, is respectfully requested.

Claim 4 stands rejected under 35 U.S.C. §112, second paragraph, as being indefinite. The Office Action holds that it is unclear how an enzyme can be selected from the group consisting of “mitochondrial proteins, apoptosis-related proteins, anti-oxidant proteins, oxidative stress proteins, and intracellular protein degradation proteins”. In response thereto, claim 4 has been amended to replace “enzyme” with “protein”. Reconsideration of the rejection under 35 U.S.C. §112, second paragraph, is respectfully requested.

Claims 6, and 31-34 stand rejected under 35 U.S.C. §112, second paragraph, as being indefinite. The Office Action holds that claim 1 requires spotting either antibodies or fluids, but claims 6 and 31-34 require spotting both antibodies and fluids. In response thereto, claim 1 has been amended to include the option of spotting both antibodies and fluids, i.e. “combinations thereof”. It is noted that the main objective of claim 1 is for spotting antibodies, and the main objective of the rejected claims is for spotting various concentrations of the antibodies to determine optimal spotting concentrations of the antibodies. No new matter has been added. Reconsideration of the rejection under 35 U.S.C. §112, second paragraph, is respectfully requested.

Claims 1 and 5 stand rejected under 35 U.S.C. § 102(b), as being anticipated by Haab (2003). Specifically, the Office Action holds that Haab teaches (a) depositing a plurality of spots of antibodies on predetermined positions of a hydrogel, (b) incubating labeled antigens/epitopes with the antibodies on the hydrogel, (c) quantifying the label at each spot in order to quantify the antigen/epitope, and (d) comparing labels at various spots. Reconsideration of the rejection under 35 U.S.C. § 102(b), as anticipated by Haab, as applied to the claims, is respectfully requested. Anticipation has always been held to require absolute identity in structure between the claimed structure and a structure disclosed in a single reference.

In Hybritech Inc. v. Monoclonal Antibodies, Inc., 802 F.2d 1367, 231 U.S.P.Q. 81 (Fed. Cir. 1986) it was stated: “For prior art to anticipate under §102 it has to meet every element of the claimed invention.”

In Richardson v. Suzuki Motor Co., Ltd., 868 F.2d 1226, 9 U.S.P.Q.2d 1913 (Fed. Cir. 1989) it was stated: “Every element of the claimed invention must be literally present, arranged as in the claim.”

Haab does not teach the step of “depositing a plurality of spots comprising antibodies selected from the group consisting of purified immunoglobulins, fluids that are unprocessed for immunoglobulin isolation, and combinations thereof” as required in presently amended claim 1, because Haab does not teach using ***purified*** immunoglobulins.

Therefore, since Haab does not disclose depositing a plurality of spots comprising antibodies selected from the group consisting of purified immunoglobulins, fluids that are unprocessed for immunoglobulin isolation, and combinations thereof as set forth in the presently pending independent claims, the claims are patentable over Haab and reconsideration of the rejection is respectfully requested.

Claims 1 and 6 stand rejected under 35 U.S.C. § 102(b), as being anticipated by Nielsen, et al. Specifically, the Office Action holds that Nielsen, et al. teaches (a) depositing a plurality of spots of antibodies on predetermined positions of a hydrogel, (b) incubating labeled antigens/epitopes with the antibodies on the hydrogel, (c) quantifying the label at each spot in order to quantify the antigen/epitope, and (d) comparing labels at various spots. Reconsideration of the rejection under 35 U.S.C. § 102(b), as anticipated by Nielsen, et al., as applied to the claims, is respectfully requested. Anticipation has

always been held to require absolute identity in structure between the claimed structure and a structure disclosed in a single reference.

Nielsen, et al. teaches spotting 0.5 mg/ml antibodies for various experiments. Nielsen, et al. does not teach “determining optimal spotting concentrations” as required in claim 6, nor the use of “fluids that are unprocessed for immunoglobulin isolation” as in claim 1. Furthermore, Nielsen, et al. does not use purified immunoglobulins just as in Haab above.

Therefore, since Nielsen, et al. does not disclose using purified immunoglobulins or determining optimal spotting concentrations as set forth in the presently pending independent claims, the claims are patentable over Nielsen, et al. and reconsideration of the rejection is respectfully requested.

Claims 1, 5, and 6 stand rejected under 35 U.S.C. § 102(b), as being anticipated by Miller, et al. Specifically, the Office Action holds that Miller, et al. teaches (a) depositing a plurality of spots of antibodies on predetermined positions of a hydrogel, (b) incubating labeled antigens/epitopes with the antibodies on the hydrogel, (c) quantifying the label at each spot in order to quantify the antigen/epitope, and (d) comparing labels at various spots. Reconsideration of the rejection under 35 U.S.C. § 102(b), as anticipated by Miller, et al., as applied to the claims, is respectfully requested. Anticipation has always been held to require absolute identity in structure between the claimed structure and a structure disclosed in a single reference.

Miller, et al. teaches spotting of 184 immunoglobulins in any **single** concentration between 0.1 and 0.3 mg/ml in quadruplicate. Miller, et al. does not teach either “determining optimal spotting concentrations” as in claim 6 or “depositing a plurality of spots comprising antibodies selected from the group consisting of purified immunoglobulins, fluids that are unprocessed for immunoglobulin isolation, and combinations thereof” as required in claim 1.

Therefore, since Miller, et al. does not disclose determining optimal spotting concentrations or depositing a plurality of spots comprising antibodies selected from the group consisting of purified immunoglobulins, fluids that are unprocessed for immunoglobulin isolation, and combinations thereof or as set forth in the presently pending independent claims, the claims are patentable over Miller, et al. and reconsideration of the rejection is respectfully requested.

Claims 1-2, 4, and 6 stand rejected under 35 U.S.C. § 102(b), as being anticipated by Haab (2001). Specifically, the Office Action holds that Haab teaches (a) depositing a plurality of spots of antibodies on predetermined positions of a hydrogel, (b) incubating labeled antigens/epitopes with the antibodies on the hydrogel, (c) quantifying the label at each spot in order to quantify the antigen/epitope, and (d) comparing labels at various spots. Reconsideration of the rejection under 35 U.S.C. § 102(b), as anticipated by Haab, as applied to the claims, is respectfully requested. Anticipation has always been held to require absolute identity in structure between the claimed structure and a structure disclosed in a single reference.

Haab teaches production of protein (antigen) and antibody (IgG) microarrays. The concentration of antigens and antibodies spotted on a slide for protein and antibody (IgG) microarrays, respectively, was any single concentration between 0.1 and 0.3 mg/ml arrayed in 6 to 12 spots for each antibody or antigen protein. Thus, there was only one concentration for each antibody spotting. For the hybridizing molecules, proteins for antibody microarrays and antibodies for protein microarrays, were diluted to various concentration (six). For example, Figure 1 shows differential levels of antigen proteins (1.6-1600 ng/ml IgG protein and 1.6-1600 ng/ml Flag protein), which were labeled with fluorescent probes and hybridized with a single concentration (0.1-0.3 mg/ml) of IgG and Flag antibodies arrayed on a slide in 6 to 12 spots for each antibody. Figure 4 shows differential

levels of antibodies (0.3-400 ng/ml), which were labeled with probes and hybridized with a **single** concentration (0.1-0.3 mg/ml) of antigens arrayed in a slide in 6 to 12 spots for each antigen. ***There was only one concentration for each antibody spotting.***

Haab does not teach “depositing a plurality of spots comprising antibodies selected from the group consisting of purified immunoglobulins, fluids that are unprocessed for immunoglobulin isolation, and combinations thereof” as required in presently amended independent claim 1.

Therefore, since Haab does not disclose depositing a plurality of spots comprising antibodies selected from the group consisting of purified immunoglobulins, fluids that are unprocessed for immunoglobulin isolation, and combinations thereof as set forth in the presently pending independent claims, the claims are patentable over Haab and reconsideration of the rejection is respectfully requested.

Claims 1-7 stand rejected under 35 U.S.C. § 102(b), as being anticipated by U.S. Patent No. 7,232,672 to Weiner, et al. Specifically, the Office Action holds that Weiner, et al. teaches (a) spotting antibodies including those from hybridomas that bind P450 onto hydrogel microarrays, (b) incubating labeled antigens/epitopes with the antibodies on the hydrogel, (c) quantifying the label at each spot in order to quantify the antigen/epitope, and (d) comparing labels at various spots. Reconsideration of the rejection under 35 U.S.C. § 102(b), as anticipated by Weiner, et al., as applied to the claims, is respectfully requested. Anticipation has always been held to require absolute identity in structure between the claimed structure and a structure disclosed in a single reference.

Weiner, et al. does not disclose the required step of depositing a plurality of spots comprising antibodies selected from the group consisting of purified immunoglobulins, fluids that are unprocessed for immunoglobulin isolation, and

combinations thereof in presently pending independent claim 1.

Therefore, since Weiner, et al. does not disclose depositing a plurality of spots comprising antibodies selected from the group consisting of purified immunoglobulins, fluids that are unprocessed for immunoglobulin isolation, and combinations thereof as set forth in the presently pending independent claims, the claims are patentable over Weiner, et al. and reconsideration of the rejection is respectfully requested.

The remaining dependent claims not specifically discussed herein are ultimately dependent upon the independent claims. References as applied against these dependent claims do not make up for the deficiencies of those references as discussed above, and the prior art references do not disclose the characterizing features of the independent claims discussed above. Hence, it is respectfully submitted that all of the pending claims are patentable over the prior art.

It is respectfully submitted that the present amendment places the application in condition for allowance as it removes all remaining issues in dispute. Specifically, the amendment does exactly what is suggested in the Office Action in order to overcome enablement rejections. The claims have been made no broader in scope thereby requiring no further searching and raising no new issues. In fact, all claims now include limitations of previously pending claims and were therefore previously searched. It is respectfully submitted that all of the claims are in condition for allowance.

In view of the present amendment and foregoing remarks, reconsideration of the rejections and advancement of the case to issue are respectfully requested.

The Commissioner is authorized to charge any fee or credit any overpayment in connection with this communication to our Deposit Account No. 11-1449.

Respectfully submitted,

KOHN & ASSOCIATES, PLLC

/Kenneth I. Kohn/

Kenneth I. Kohn, Reg. No. 30,955

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Dated: December 7, 2010

CERTIFICATE OF ELECTRONIC FILING VIA EFS-WEB

Date of Electronic Filing: December 7, 2010

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